

## ENVIRONMENTAL IMPACT ON PRODUCTION OF ALKALOIDS IN TULSI (*OCIMUM SANCTUM L.*) AND ARDUSI (*ADHATODA VASICA NEES.*) AS INFLUENCED BY SEASONAL VARIATION

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### ABSTRACT

Medicinal plants from a part of our daily life. Some of these herbs are consumed regularly by us. *Ocimum sanctum L.* (Tulsi) of family Labiatae and *Adhatodavasica Nees.* (Ardusi) of family Acanthaceae are commonly used to control a cough and cold. Alkaloids constitutions are the photoactive components of these plants. The present study focuses on the investigation of alkaloids contents of these important medicinal plants harvested in different seasons. It was observed that among the two varieties of Tulsi (Black and White) the white tulsi showed more active components accumulation during winter season whereas notable variation was not recorded in Ardusi. Contents of the alkaloids were influenced by the environment.

**KEYWORDS:** *Ocimum Sanctum L.*, *Adhatoda Vasica Nees.*, Alkaloids, Medicinal Plants & Environmental Effect

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### INTRODUCTION

The fact that almost every plant has a medicinal value is a major constraint in categorizing plants into medicinal and non-medicinal ones. The uses of plant in the traditional medicine system of many other cultures have been extensively documented. These plants continues to play an essential role in health care and it has been estimated by the world health organization that approximately 80 percent of the world's inhabitants rely mainly on traditional medicine for their primary health care. <sup>1,2</sup> Some of these herbs are consumed regularly by us. *Ocimum sanctum* Linn. -sacred basil (Tulsi) of family *Lamiaceae* and *Adhatoda vasica* Nees (Ardusi) of family *Acanthaceae* are commonly used to control cough and cold.

#### Tulsi

*Ocimum sanctum* Linn. belonging to family *Lamiaceae* is one of the most astonishing herbs for having enormous medicinal potentialities. It is widely used in Indian system of medicine and is commonly known as Tulsi or Holy Basil and is known as "the queen of herbs". The genus *Ocimum* comprises between 60 to 150 species of herbs and shrubs which are naturally found wild in the tropical and subtropical regions of the world. <sup>3</sup> In India, the plant is grown throughout the country from Andaman and Nicobar islands to the Himalayas up to 1800 meters above the sea level. <sup>4</sup>The leaves of the plant are considered to be very holy and often form a consistent part of the Hindu spiritual rituals (Tirtha or Prasada). *Ocimum sanctum* L. has two varieties i.e. black (Krishna Tulsi) and

green (Rama Tulsi), their chemical constituents are similar. Both the varieties also have common medicinal properties.<sup>5</sup>

### **Ardusi**

*Adhatoda vasica* Nees. belonging to family *Acanthaceae*, commonly known as Adosa, is a small, evergreen shrub found in many regions of India and throughout the world, with a multitude of uses in traditional Ayurveda. *A. Vasica* is the most well-known for its effectiveness in treating respiratory conditions. The leaves of *A. vasica* shows stimulant effect on the respiratory system. *A. vasica* shows an antispasmodic and expectorant effect and has been used for centuries with much success to treat asthma, chronic bronchitis, and other respiratory conditions. The powdered herb, boiled with sesame oil, is used to heal ear infections and arrest bleeding. Boiled leaves are used to treat rheumatic pain, and to relieve the pain of urinary tract infections. It is also believed to have abortifacient properties. It is used in some parts of India to stimulate uterine contractions, thus speeding childbirth.<sup>6</sup>

Plants are constructed with the help of plant “Chemical bricks” and these are derived from plants as natural products for different purpose. Among the natural products few act as foundation stones and set the frame work of plant life and other are those synthesized from the product in order to maintain various metabolic activities have been named primary and secondary product respectively. The Primary metabolic pathway is common to all life forms and some of reactions lead to the formation of compounds unique to a few species. These reaction are classified under the terms “Secondary metabolism” and their product are known as “Secondary Metabolites”. These substances include alkaloids, antibiotics volatile oil and resins, tannins, flavonoids etc.<sup>7</sup>

Alkaloids, flavonoids, phenolics, terpenoids, and essential oils are some of the important bioactive phytochemicals.<sup>8</sup> Alkaloids constituents are the photoactive componenets of Tulsi and Ardusi. Alkaloids are a group of nitrogen-containing bases. Most of them are drugs. Only few (like caffeine) are derived from purines or pyrimidines, while the large majority is produced from amino acids. Alkaloids are a class of compounds that typically contain nitrogen and have complex ring structures.<sup>9</sup> It is mostly unknown why plants produce alkaloids. Various theories have been proposed to explain their existence.<sup>10</sup> Some suggest that alkaloids are byproducts of normal plant metabolism. It is also thought that alkaloids may provide a means of defense against insects and animals. Alakoids may also be reservoir for molecules that plants often use.<sup>11</sup>

The physiological effects of alkaloids have made them important compounds in medicine. They have been used as painkillers, stimulants, muscle relaxers, tranquilizers and anesthetics.<sup>12</sup> The four types of alkaloids that have the most important economic impact include opiates, cocaine, caffeine and nicotine.<sup>13</sup> The production of metabolites in the plants may vary with respect to the change in surrounding environment due to seasonal change.<sup>14, 15</sup> These change can be investigated by analyze the plant materials during different seasons.

## **MATERIALS AND METHODS**

### **Plant Material**

For the present investigation fresh leaves of *Ocimum sanctum* (L.) and *Adhatoda vasica* Nees. were collected from Botanical Garden of M. G. Science Institute, Ahmedabad. The collected plant material was taxonomically identified by using renowned floras.<sup>16</sup> Fresh leaves were collected in two different season viz., summer and winter. The fresh leaves were thoroughly washed with tap water. They were dried at room temperature. Dried leaves were powdered using mortar pestle. The powder was successively extracted with different solvent.

### Extraction Method

Powder of 10 gm of dried leaves was added to 80 ml of 10 % ammonical methanol, heated at 65 °C cold extracts with occasional shaking and kept overnight at room temperature. The extract was then filtered and again 80 ml of 10% ethanol was added and procedure was repeated one more time for extraction of alkaloids from the leaves. The combined extract was allowed to dry. A greenish viscous extract was obtained. During analysis dry content was redissolved in methanol.<sup>17</sup>

### Separation Method

Analytical and preparative thin layer chromatography was performed on silica gel plates (60 F 254 or glass plates coated with silica gel G 60) with chloroform as solvent system. In some experiments, BHT and BHA (0.01gm/ml) was added to chloroform. Chromatograms were visualized under ultra violet light.<sup>17</sup>

- Preparing thin layer (e.g. silica gel G.)
- Choice of solid support (e.g. glass plate)
- Sample application
- Choice of solvent (mobile phase)
- Development of chromatograph
- Detecting or spraying reagent
- Identification and calculation of R<sub>f</sub> value

$$R_f = \frac{\text{Distance travelled by sample}}{\text{Distance travelled by solvent}}$$

### TLC Condition

<b>Extract:</b>	Methanolic extract
<b>Solvent System:</b>	Methanol : Ethyl acetate (1 : 9)
<b>Derivatizing Agent:</b>	1 % Ceric ammonium sulphate and Iodine gas

### Effect of Addition of BHT ant BHA

The extract was stored in vials in refrigerator. However, during chromatography and subsequent chromatograms, it was observed that the number of bands slowly increased with time. The appearance of new bands was suspected to be due to oxidation. To avoid oxidation, we added BHT or BHA to some samples.

## RESULTS AND DISCUSSIONS

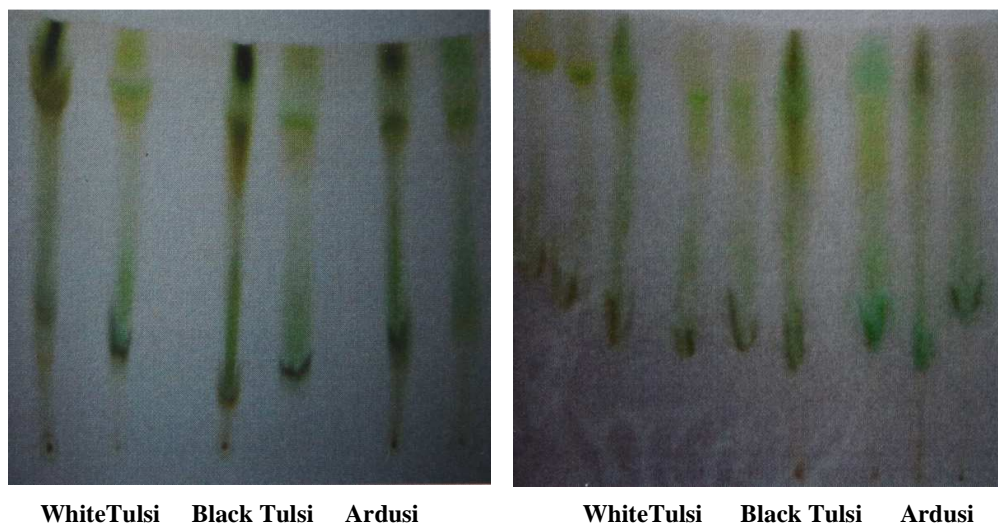
Methanol extract obtained from sample leaves was carried out to thin layer chromatography in order to establish the purity and composition of materials. The purified components of methanol extract of Tulsi and Ardusi leaves were identified by iodine test. The alkaloids present in the leaves are well extracted through 10 % methanol. During separation, large variation was observed in the production of alkaloids as influenced by two different seasons. In white tulsi, during summer there were 7 spots detected while in winter it showed increase in the alkaloid content. It showed 10 spots in winter

season.

In black tulsi least variation was observed. In summer it showed 6 spots of alkaloids while in winter it showed 5 spots. In ardusi the alkaloid content was higher in summer than that in winter. It showed 8 spots in summer while 5 during winter season. The overall observation revealed that highest numbers of alkaloids were observed during winter season in white tulsi.

**Table 1: Comparison of Rf values of the Three Plants in Two Different Season**

Sr. No.	Rf Values					
	White Tulsi		Black Tulsi		Ardusi	
	Summer	Winter	Summer	Winter	Summer	Winter
1	0.07	0.05	0.14	0.16	0.11	0.20
2	0.14	0.18	0.37	0.30	0.21	0.30
3	0.21	0.21	0.44	0.52	0.28	0.43
4	0.28	0.23	0.53	0.75	0.41	0.73
5	0.39	0.28	0.82	0.87	0.52	0.86
6	0.77	0.46	0.93	-	0.82	-
7	0.85	0.67	-	-	0.89	-
8	-	0.76	-	-	0.93	-
9	-	0.83	-	-	-	-
10	-	0.90	-	-	-	-



**Figure 1: TLC Analysis in Summer**

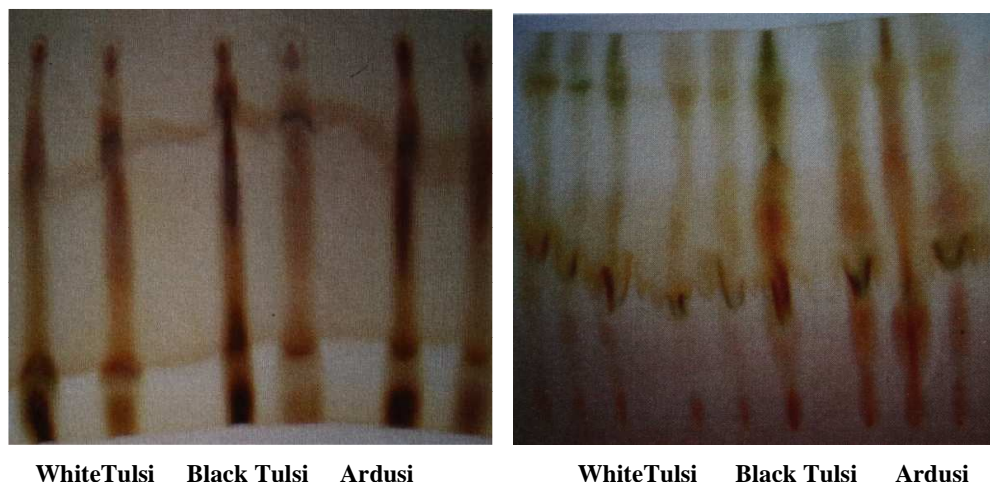


Figure 2: TLC Analysis in Winter

## CONCLUSIONS

The white tulsi showed more active components accumulation during winter season as black tulsi and ardusi showed more alkaloids during summer season. Alkaloids content of these three plants are influenced by the change in environment due to different seasons.

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